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TRANSMUTATIONS WITHIN THE STREPTOCOCCUS-PNEUMOCOCCUS GROUP.*

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(WITH PLATE 1.)

Changes in fermentative, morphological, and other properties of certain streptococci, particularly the hemolytic streptococcus, have been noted by Ruediger,[1] Anthony,[2] Walker,[3] and especially by Davis[4] and by Buerger and Rittenberg.[5]

Davis concludes that the "transformation of one member into another within certain limits appears to be not an uncommon phenomenon." Buerger and Rittenberg converted atypical pneumococci ("streptococcus cultural type") into typical pneumococci by animal passage. They were unable, however, to convert typical hemolytic streptococci into pneumococci and vice versa. Davis and Rosenow have shown that the encapsulated streptococcus from "septic sore throat" can be converted into *Str. mucosus* on the one hand and hemolytic streptococcus on the other. Hemolytic streptococci when soaked in unheated cows' milk, obtained in a

* Received for publication September 19, 1913.

[1] *Jour. Infect. Dis.*, 1906, 3, p. 663.

[2] *Ibid.*, 1909, 6, p. 332.

[3] *Proc. Royal Soc.*, 1911, S.B. 83, p. 541.

[4] *Jour. Infect. Dis.*, 1913, 12, p. 386.

[5] *Ibid.*, 1907, 4, p. 609.

sterile manner,[1] take on cultural and other features resembling streptococci obtained from cases during a milk epidemic of sore throat, and when later passed through animals they take on the features of *Str. mucosus*.

Beattie and Yates[2] were unable to obtain permanent, specific fermentative reactions for streptococci. Levy[3] was unable to separate certain strains of *Str. viridans* from hemolytic streptococcus and considers *Str. mucosus* a variety of pneumococcus. Heinemann[4] has shown that "*Str. lacticus*," by animal passage, may take on features similar to hemolytic streptococci. Koch and Pakschischewsky[5] have shown that certain equine streptococci (Druse-Streptococcus), while not identical in all respects, resemble very closely indeed human virulent *Str. longus*.

In previous papers[6] I have shown that *Str. viridans* isolated chiefly from the blood in cases of subacute endocarditis and obtained also from the throat and other sources may by animal passage take on the properties of typical pneumococci; and hence I designated them as modified pneumococci. During a study on autolysis of pneumococci in salt solution and the effect of sodium oleate and bile on virulent pneumococci their transformation into hemolyzing streptococci was observed.

The anaphylactic reaction indicates that the chemical compositions of the various members of the group are similar. I have shown[7] that extracts of hemolytic streptococcus sensitize guinea-pigs to extracts of the pneumococcus and *Str. mucosus*, and vice versa. Davis,[8] working with heated suspensions of the various organisms, recently had similar results and concludes that the anaphylactic reaction is of little value in differentiating the various members of the streptococcus group.

It should be pointed out here, however, that pneumococci and hemolytic streptococci, for example, call forth other immunity reactions (opsonins and agglutinins) which are specific and which serve to separate them into distinct classes or types.

[1] Rosenow, *Jour. Infect. Dis.*, 1912, 11, p. 338.
[2] *Jour. Path. and Bact.*, 1911, 16, p. 246.
[3] *Virch. Arch.*, 1907, 157, p. 327.
[4] *Jour. Infect. Dis.*, 1907, 4, p. 89.
[5] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1913, 74, p. 1.
[6] *Jour. Infect. Dis.*, 1909, 6, p. 245; *ibid.*, 1910, 7, p. 411.
[7] *Ibid.*, 1911, 9, p. 190.
[8] *Ibid.*, 1913, 12, p. 386.

Modifications in the human subject have also been observed. Buerger and Rittenberg found that the pneumococci obtained from the blood in puerperal sepsis are quite different from those obtained from metastatic abscesses in the same case. I have made similar observations in acute otitis and in pneumonia[1] that suggest the transformation *in vivo* of *Str. mucosus* and of pneumococcus into hemolytic streptococcus. Toenniessen[2] has produced mutational forms in case of *B. mucosus*. It does not appear, however, that it has been shown that typical hemolytic streptococci from scarlet fever, for example, can be converted into typical pneumococci. The "pure line" requirement—that is, working with a strain obtained from single individuals—of students of heredity has not been met except in the case of the transformation of *Str. viridans* into pneumococcus as mentioned.

A more elaborate study of this question, therefore, seemed desirable.

In the experiments now to be described, growth on the surface of human blood agar was used as a criterion of change. The standard agar was prepared from Liebig's extract of beef and Witte's peptone; approximately 0.5 c.c. of sterile defibrinated human blood was added to each tube of 7 c.c. of melted and cooled agar just before pouring the plates.

EXPERIMENTS WITH VARIOUS STREPTOCOCCAL STRAINS.

July 28, 1911. Strain 595 was isolated from the tonsil in acute tonsillitis of scarlet fever. A practically pure culture of hemolyzing streptococci was obtained on blood agar plates. One typical hemolyzing colony was selected and plated again and a single colony from the second plating used for the subsequent work. From this date until June, 1912, it was grown on human blood agar. During this time it was plated out three times, and it always yielded only hemolyzing colonies, and the growth on blood agar slants always caused hemolysis. It produced arthritis in rabbits.

June 29, 1912. Subculture was made on blood agar and the tube sealed with a cork previously soaked in paraffin.

October 8. Culture on blood agar plates, hemolyzing colonies only. The amount of hemolysis was approximately one-third as great as when first isolated. Inoculations were made from single colonies on two blood agar slants containing peptone and extract of beef. These uncorked tubes were kept at 37° C. until November 6. On this date the media were very dry. Inoculations on the usual blood agar slants were made and placed at 37° C.

[1] *Jour Infect. Dis.*, 1911, 8, p. 500

[2] *Centralbl. f. Bakteriol.* I, *Orig.* 1913, 6, p. 391

November 15. Subcultures on the surface of blood agar plate the day before yielded two kinds of colonies, one larger, producing the usual zone of hemolysis, the other smaller and producing no hemolysis. Subcultures were made on the surface of blood agar plates from the two types of colonies.

November 17. The larger colonies yielded only hemolyzing colonies while the smaller yielded only small, green, tightly adherent colonies with no trace of hemolysis. Subcultures of each were made on the surface of Löffler's serum and four colonies of each variety plated on blood agar plates.

November 19. The subcultures of each of the four hemolyzing colonies yielded only hemolyzing colonies. Three of the adherent colonies yielded only adherent green colonies, while the other yielded adherent and non-adherent green colonies in about equal proportions, but no hemolyzing colonies. Duplicate cultures on blood agar slants from a single hemolyzing and a single adherent green colony.

November 21. Hemolyzing colony yielded hemolyzing growth, the green colony a non-hemolyzing, adherent, green growth. Subcultures of each were made on Löffler's serum and blood agar slants, sealed and placed at 37° C.

The hemolyzing strain produced a diffuse turbidity in ascites broth together with some sediment, the green-producing strain, no turbidity but an abundant sediment. Smears of the hemolyzer showed diplococci and short chains, of the green-producer, both large and small diplococci and very long chains and clumps. Any diplococcus arrangement in the chains of the hemolyzer was hard to make out (Fig. 1); in the green-producer the diplococci were very distinct, elongated, and often lanceolate in shape (see Fig. 2). Neither strain fermented inulin. The hemolyzer fermented mannite, the green-producer did not; the former failed to ferment maltose and saccharose, the latter did; both precipitated serum-dextrose agar, the hemolyzer more markedly; neither produced acid in pneumonic serum; neither was soluble in rabbit bile.

If the modified strain which adhered to the surface and produced the green zone around the colonies was really *Str. viridans*, as seemed likely, then it should have produced characteristic lesions in animals. Accordingly, on November 19, the growth from 20 to 60 c.c. of ascites-dextrose broth suspended in NaCl solution was injected in the ear vein of four small rabbits (600 to 750 gms.). Two other rabbits were injected with comparable doses of the corresponding hemolyzing strain. Previously I have shown that the ability of *Str. viridans* to produce valvular hemorrhages and endocarditis depends definitely on the property to form long chains and clumps. Hence two of the four rabbits were injected with a thoroughly shaken suspension in which only small clumps of organisms could be found in smears and two with the clumped suspension the result of prolonged centrifugation. All the rabbits died within 36 hours. The two injected with the shaken suspension showed small tricuspid hemorrhages but no hemorrhages anywhere else. The two injected with the clumped suspension showed large tricuspid and papillary hemorrhages, and hemorrhages in the glomerular tufts, and one showed hemorrhages in the mucous membrane of the pyloric end of the stomach. The two injected with the hemolyzing strain did not show valvular hemorrhages but subendocardial hemorrhages of the septum in the left ventricle. Cultures from the blood of the four injected with the green-producer showed a few green colonies in three; the joints in two showed a few green colonies while in the others they were sterile. The two injected with the hemolyzing variety showed a large number of hemolyzing colonies from the joints and a moderate number from the blood. Subcultures of each were made on blood agar slants.

The effect of clumping and the affinity for the heart valves of the green-producing variety and for joints of the hemolyzing variety are well illustrated by this experiment. In this case the transformation of a hemolyzing streptococcus to a green-producing streptococcus occurred on blood agar with peptone and beef extract, the supply of oxygen being abundant.

There now follows an illustration of another method by which transformation may be brought about.

January 29, 1913. A blood agar plate which was inoculated with the growth of hemolytic streptococcus (No. 683) became contaminated accidentally with three colonies of what was apparently *B. subtilis*. The plate contained mostly hemolyzing colonies but there was found in addition a number of green, moderately adherent colonies. These were all in relatively close proximity to the colonies of the bacilli. Smears of the green colony showed small gram-staining diplococci, often in clumps and chains. The large colonies were large, gram-staining bacilli.

Subcultures were now made on blood agar plates of two hemolyzing and two green colonies and from two of the bacillary colonies.

January 31. The green colonies produced green colonies, the hemolyzing colonies, hemolyzing colonies, and the *B. subtilis* plate showed no green nor hemolyzing colonies.

February 5. Subcultures of the hemolyzing and green-producing colonies of Strain 595 were made on the surface of blood agar plates, one of which was inoculated also with a dilute culture of *B. subtilis*.

February 6. The plates containing the hemolyzing strain showed only hemolyzing colonies, while the set of this strain inoculated also with *B. subtilis* showed both hemolyzing and green, adherent colonies; the latter exclusively in a zone within a radius of approximately 0.5 cm. around the *B. subtilis* colonies. Approximately one-half of the colonies inside of this zone were green, adherent, and non-hemolyzing.

Subcultures of the non-hemolyzing strain which was made on November 21, 1912, on blood agar still yielded only green colonies while those from Löffler's serum yielded three-fourths green and one-fourth hemolyzing colonies.

In order to make it even more certain that the green colonies on the *B. subtilis* plate were not accidental, four hemolyzing colonies were plated on the surface of blood agar in such a way that one-half of the plate contained only streptococcus colonies while the other half contained *B. subtilis* as well. In the same way subcultures were also made from blood agar slants and this time with a strain of *B. subtilis* which had been plated out from single colonies four times and had always yielded only *B. subtilis*. The hemolyzing colony and the green colony on the surface of blood agar slants bred true. Each hemolyzing colony gave only hemolyzing colonies on the half of the plate containing no *B. subtilis* while approximately two-thirds of the colonies in the other half were adherent, green colonies. The part of the plates made from the blood agar slants without *B. subtilis* yielded only hemolyzing colonies; the parts inoculated with both yielded both varieties of colonies. The green colonies again appeared in rather close proximity to *B. subtilis* colonies.

The following experiments illustrate the affinity of the two varieties of streptococci after one animal passage for the valves of the heart and the joints respectively.

Rabbits were injected in the ear vein. The organisms, grown in ascites-dextrose broth, were sedimented, the broth poured off, and the organisms suspended in NaCl solution so that 1 c.c. represented 10 c.c. broth culture.

Rabbit 221 (535 gms.).—

November 26. Injected with the growth from 25 c.c. of broth of the green-producing strain after one animal passage.

November 27. Seemed quite well.

November 28. Killed; no gross lesions except two hemorrhages in tricuspid valve. Smears from blood and joints negative. Blood agar plate cultures from blood, knee joints, and from surface of valve all sterile. From the tissue of the valve about 150 green colonies developed.

Rabbit 322 (630 gms.).—

November 26. Injected with the growth from 40 c.c. of broth of the green-producing strain after one animal passage. Some dyspnea soon after injection.

November 27. Seemed quite well.

December 1. Seemed ill.

December 3. Loss of weight, had grown blind from an opacity and turbidity of anterior chamber due to conjunctivitis. Chloroformed. Large vegetative tricuspid endocarditis; multiple, white miliary nodules in cortex of kidney. Smears from vegetations, from renal lesions, and anterior chamber of eye show many gram-staining diplococci and clumps. Blood and joint fluids sterile. Large number of green-producing colonies developed from vegetation, from kidneys, and from the anterior chamber.

Rabbit 323 (575 gms.).—

November 26. Injected with growth from 40 c.c. of broth of the hemolyzing strain after one animal passage. Slight dyspnea soon after injection.

November 27. Dead. No gross lesions but some fluid in peritoneal cavity containing hemolyzed blood. Large number of hemolyzing colonies from blood and moderate number from joints and peritoneal fluid.

Since it has been shown that the usual viridans-strains do not produce suppuration when injected subcutaneously and do not cause diffuse peritonitis when injected intraperitoneally, it was thought worth while to test the properties of these two strains in this respect. Four guinea-pigs were injected with comparable doses of the green-producing and hemolyzing varieties before animal passage. The green-producer disappeared rapidly and the animals recovered, while the hemolyzer increased in the blood and caused death with general peritonitis.

From these experiments it would seem, then, that the green-producer really was *Str. viridans*. If this was true, then animal passage should have converted it into a pneumococcus just as I have shown to be the case with *Str. viridans* obtained from human sources. A strain from a single colony from one of the rabbits injected November 19 was selected for this purpose. In order to guard against the loss of the organism in the animals and the possibility of obtaining pneumococci from sources in the animal other than the organisms injected, the first passages were

carried out in duplicate, the animals from absolutely healthy stock were caged separately, and blood agar plate cultures were made from the blood and peritoneal exudate at intervals during life and as soon after death as possible.

Guinea-pig 993.—

November 23. Injected intraperitoneally with the growth from 40 c.c. of ascites-dextrose broth of the strain from Rabbit 595-G^2.

November 24. Dead; mild serofibrinous peritonitis. Smears from blood showed no organisms while those from peritoneal exudate showed many leukocytes, diplococci, and chains. There was marked phagocytosis. Cultures from the blood and peritoneal exudate; 1.5 c.c. of the latter were injected intraperitoneally in Guinea-pig 996.

November 25. Cultures from blood showed a few while those from the peritoneal exudate showed many green colonies in pure growth. Guinea-pig 996 dead; serofibrinous peritonitis and pleuritis. Peritoneal smears showed more marked phagocytosis than those from the pleural cavity. Cultures made and 1 c.c. of peritoneal exudate injected intraperitoneally in Guinea-pig 997.

November 26. Cultures from blood, peritoneal and pleural fluids, gave a pure culture of green-producers, the colonies being distinctly larger and more moist than before inoculation, but without capsules.

November 27. Guinea-pig 997 dead; serofibrinous peritonitis. Smears from the exudate showed less phagocytosis, few chains, fewer leukocytes; a few diplococci found in the blood. Pure culture of green colonies from blood and peritoneal fluid. Subcultures from the blood on surface of blood agar slant and in ascites-dextrose broth gave growths that on November 29 appeared green on transmitted light and quite moist; capsule stain negative. In the broth diffuse turbidity with some sediment, consisting entirely of non-encapsulated diplococci and short chains. Fresh subcultures on the surface of blood agar slants, in inulin broth and on inulin agar, on ascites-dextrose agar and in pneumonic serum.

Guinea-pig 1003.—

November 30. Injected intraperitoneally with the surface growth of one blood agar slant.

December 1. Seemed ill.

December 2. Dead; serofibrinous peritonitis and pleuritis. Smears gave diplococci in rather large numbers in the blood, some of which were distinctly encapsulated, and very many in the peritoneal and pleural exudate. Phagocytosis now much less than in the previous animals. Cultures from the blood on the surface of blood agar slant and plate and on the latter from the peritoneal and pleural exudate.

The strain from Guinea-pig 997 (595-G^4) ferments inulin broth, no longer precipitates ascites-dextrose agar, but does not produce acid in pneumonic serum as do typical pneumococci.

Guinea-pig 1007.—

December 3. Injected intraperitoneally with the surface growth of one blood agar slant obtained from the blood of Guinea-pig 1003.

December 4. Dead; serofibrinous peritonitis, pericarditis, and pleuritis. Smears gave encapsulated diplococci in exudate and blood. Very little phagocytosis. Subcultures in the usual way.

Similar experiments were repeated until the strain had been passed through 14 animals. It had now become so virulent that 0.1 c.c. of a broth culture killed in 24 hours by producing bacteremia. At this time the organisms were found encapsulated in the blood, in the exudate as well as on blood agar and in ascites broth cultures. Morphologically they were indistinguishable from typical pneumococci (see Fig. 3). The strain now fermented inulin broth and agar, did not cloud ascites-dextrose agar, produced acid in pneumonic serum (see Table 1), was soluble in bile, and autolyzed, as pneumococci do, in NaCl solution under ether. Its broth-culture filtrate behaved like pneumococcus broth-culture filtrates and while the strain failed to grow in pneumococcus broth-culture filtrates it grew readily in streptococcus broth-culture filtrates (Marmorek's test). Table 1 shows the fermentative and other cultural characteristics of Strain 595 as a streptococcus and as a pneumococcus on March 11, 1913. Similar results have been obtained at other times.

TABLE 1.

THE FERMENTATIVE AND OTHER FEATURES OF STRAIN 595 AS A STREPTOCOCCUS AND AS A PNEUMOCOCCUS.*

MEDIA	STRAIN 595					
	As Streptococcus			As Pneumococcus		
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
Blood agar	H	H	H	G	G	G
Serum dextrose agar	+	+	++	o	o	o
Inulin broth	o	o	o	+	+	++
Inulin agar	o	o	o	o	+	+
Pneumonic serum	o	o	o	+	+	+
Mannite	o	o	o	o	o	o
Saccharose	o	o	+	+	+	+
Dextrose	+	+	+	+	+	+
Lactose	o	o	+	o	o	o
Maltose	o	o	o	o	o	+

* In the various tables, H stands for hemolysis and G for green. + stands for clouding of serum dextrose agar, acid production in pneumonic serum, and for fermentation of the various sugars. o stands for no clouding of serum dextrose agar, no acid production in pneumonic serum, and no fermentation of the various sugars.

The original hemolyzing strain has been grown on blood agar continuously and has remained unaltered. The strain as a pneumococcus has also been continuously grown on blood agar slants with cotton plugs. It has remained virulent and as a pneumococcus in the cultures; transfers have been made once or twice a week

for six months. However, a subculture made five months after the transformation had taken place from an old blood agar slant which had become quite dry from evaporation, yielded a hemolytic, relatively avirulent streptococcus similar to the original growth. The fermentative and other cultural properties of this strain after reversion from pneumococcus were the same as before its transformation into a pneumococcus except that it now fermented mannite and maltose. It had lost its capsule and again become susceptible to phagocytosis by human leukocytes in human serum. Its ability to grow in the blood stream and to kill by streptococcemia had disappeared while the affinity for joints had returned.

Two other important alterations have been observed in this strain. The strain as a pneumococcus after 14 animal passages, and two other strains of pneumococci and two of streptococci were inoculated February 7 into two sets of tubes containing dialyzed beef serum agar slants. To these there were added varying amounts of Ringer's solution. One set was placed at 37° C., the other kept at room temperature. Subcultures on the surface of blood agar plates were made at the end of 10 days. No noteworthy changes in the character of growth on blood agar were noted in the tubes made isotonic with two and five times the strength of normal Ringer's solution but in the tubes containing 10 times the normal of Ringer's solution at 37° C. this and one of the other strains of pneumococcus, namely No. 683, had taken on the characteristics of *Str. viridans*, and in the tubes containing 20 times the strength of normal Ringer's solution, at room temperature, one streptococcus had become altered so that subcultures yielded very small pin-point colonies surrounded by a wide zone of hemolysis, the smears showing very small gram-staining diplococci and chains exactly similar to certain strains of streptococci obtained not infrequently from the crypts of extirpated tonsils. Cultures were made from three colonies on the surface of blood agar plates. Each yielded only the small colonies and cocci.

If Strain 595 was really a streptococcus and the one formed from it really a pneumococcus, then the former should have produced specific antibodies for streptococci and the latter specific antibodies for pneumococci.

In order to test this point two rabbits, Nos. 458 and 459, weighing 750 gms. each, were injected intraperitoneally, April 3, with the heated (60° C. 30 min.) suspensions in NaCl solution from two blood agar slants, Rabbit 458 with the streptococcal strain, and Rabbit 459 with the pneumococcal strain. Samples of blood from each were taken just before injection, for three consecutive days, and on the fifth day following the injection. The sera were kept frozen in a vacuum bottle until April 9 when the opsonic index was determined as indicated in Charts 1 and 2. The counts were made by individuals without knowledge of the dates when the sera were obtained or the

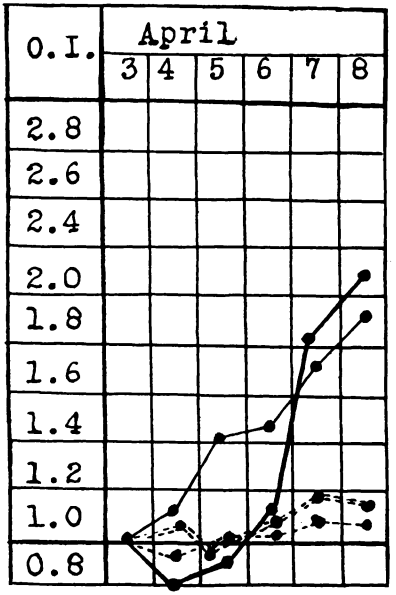

CHART 1.—Opsonic index of serum of rabbit immunized with Strain 595 as a streptococcus.

Homologous streptococcus.
Heterologous streptococcus.
– – – – Homologous strain as pneumococcus.
= = = = Heterologous pneumococcus.

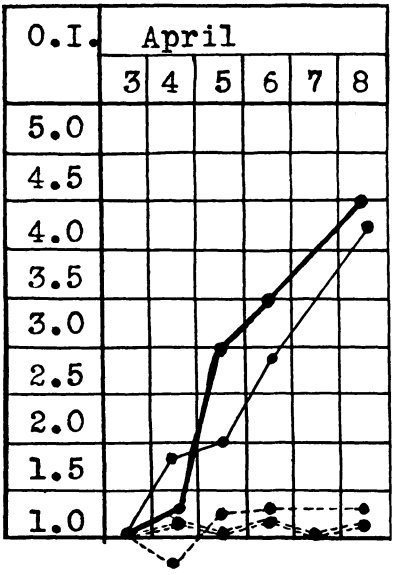

CHART 2.—Opsonic index of serum of rabbit immunized with Strain 595 as a pneumococcus.

Homologous pneumococcus.
Heterologous pneumococcus.
– – – – Homologous strain as streptococcus.
= = = = Heterologous streptococcus.

strains used. The charts show that the strain as a streptococcus caused a specific increase in opsonin for homologous and one other strain of hemolytic streptococcus, but no perceptible increase for the homologous strain as a pneumococcus nor for another strain of pneumococcus. The strain as a pneumococcus on the other hand caused a specific rise in opsonin for both the homologous and another strain of pneumococcus but not for the homologous strain as a streptococcus nor for another streptococcus. In order to check these results the rabbits were injected with comparable doses (growth from two blood agar slants) on April 15, 17, and 18, and were then bled on April 26. The point of opsonic extinction of the serum of each and a normal rabbit as control was now determined. The serum from the rabbit immunized with Strain

595 as a streptococcus gave opsonic extinction for the homologous and another strain of hemolytic streptococcus at a dilution of 1–192; for Strain 595 as a pneumococcus and for another pneumococcus at 1–12. The serum from the rabbit immunized with Strain 595 as a pneumococcus showed opsonic extinction at a dilution of 1–192 for the homologous and the other strain of streptococcus at 1–48. The serum from the normal rabbit gave opsonic extinction for the streptococci at 1–48 and for the pneumococci at 1–12. Counts were made also of the cocci taken up in the various dilutions, 50 leukocytes being counted. The results, given in Table 2, the opsonic index, and the point of opsonic extinction show that the serum from the rabbit immunized with Strain 595 as a hemolytic streptococcus contained an increase in specific opsonin for streptococci and the serum from the rabbit immunized with Strain 595 as a pneumococcus an increase in specific opsonin for pneumococci.

TABLE 2.

THE OPSONIC POWER OF THE SERUM OF NORMAL AND IMMUNIZED RABBITS.

SERUM	NUMBER OF ORGANISMS TAKEN UP IN THE VARIOUS DILUTIONS IN DETERMINING THE POINT OF OPSONIC EXTINCTION			
	Streptococcus 595	Streptococcus M	Pneumococcus 595	Pneumococcus 678[19]
Rabbit immunized with Strain 595 as a streptococcus	112	159	8	6
Rabbit immunized with Strain 595 as a pneumococcus	65	82	45	26
Normal rabbit control	59	76	6	7

Strain XII was isolated as a typical hemolytic streptococcus from a single colony on a blood agar plate (June 2, 1912) inoculated with the blood of a guinea-pig which died from a streptococcemia 48 hours after the injection of a suspension of separator slime. The growth from one blood agar slant was injected intraperitoneally into a rabbit which died in 24 hours with peritonitis. The blood agar plate inoculated with the blood showed a pure culture of markedly hemolyzing colonies. A subculture from a single colony was made and the strain cultivated continually on blood agar slants which were closed with paraffin corks until January 19, 1913. From blood agar plates now made only hemolyzing colonies were obtained. A single colony was used in inoculating two blood agar slants. One strain has been cultivated on blood agar in the usual way continuously since. It has been plated out on blood agar repeatedly and has always produced only hemolyzing colonies. The amount of hemolysis, however, is approximately one-half as great as it was at first. The other strain was used for this work. By growing it for three generations on ascites-dextrose agar in pure oxygen and then plating on blood agar plates three types of colonies were obtained: approximately one-half of the colonies produced a slight and hazy hemolysis; one-third were grayish, non-adherent colonies which did not affect the media; and then there were some green, tightly adherent colonies. The slightly hemolyzing colonies showed diplococci and short chains, the gray colonies small micrococci and an occasional short chain. These growths closely corresponded, culturally and morphologically, to two types of the organisms obtained from rheumatism. The adherent green colonies showed long chains and clumps of small elongated diplococci similar to those shown in Fig. 2. Plate cultures from three of each of these varieties showed that each bred true and a single colony of the green variety was selected for animal passage.

The growths from 20 to 60 c.c. of ascites-dextrose broth were injected intravenously successively in five rabbits. Valvular hemorrhages were found in two after two and three passages respectively, and vegetative endocarditis in two, in the fourth and fifth passage. The organism was now injected intraperitoneally in guinea-pigs, and cultures were made on blood agar plates after death or after 48 hours if recovery seemed likely. After three passages in this way the organism had become quite virulent, producing peritonitis, and had most of the characteristics of a pneumococcus but had no demonstrable capsule and still formed chains. It now fermented inulin, produced acid in pneumonic serum, no longer clouded ascites-dextrose broth, autolyzed in NaCl solution, and dissolved in bile. After 12 animal passages its virulence was so great that 0.1 c.c. of ascites-dextrose broth culture injected intraperitoneally killed a guinea-pig in 16 hours. The blood and cultures now showed organisms with a wide, stainable capsule that corresponded to all the cultural and agglutination tests of pneumococci. After 14 animal passages the capsule had become still more marked, the growth on blood agar and ascites-dextrose agar was now mucoid in character, and the organism corresponded to *Str. mucosus* in every respect. At first the guinea-pigs had a rather mild peritonitis, later a marked serofibrinous peritonitis and pleuritis and still later also pericarditis, when as a *Str. mucosus* the exudates were mucoid in character. After five rabbit (intravenous injection) and two guinea-pig (intraperitoneal injection) passages it produced death in a rabbit in 22 days from a general anasarca due to an obstructive vegetative endocarditis of the tricuspid valve, cultures from the vegetation yielding an organism resembling in every way *Str. viridans*. After nine animal passages its affinity for the endocardium had disappeared and it now produced pulmonary hemorrhages and acute peribronchial lymphadenitis on intravenous injection in rabbits. This strain has been cultivated continuously on blood agar for six months, making transfers once or twice a week. It has lost its mucoid character but has retained its capsule, virulence, and the other characteristics of a pneumococcus.

The hemolyzing variety of this strain was converted into *Str. viridans* also by growth in oxygen, by growth in symbiosis with *B. subtilis*, in salt free broth, and in hypertonic broth.

Strain 734 was isolated January 27, 1913, from the joint of a case of acute articular rheumatism. This organism as isolated produced long chains in broth (Fig. 4), green on blood agar, did not ferment mannite nor inulin, and when injected intravenously in rabbits produced multiple arthritis, endocarditis, and pericarditis. In an attempt to modify this strain it was inoculated (February 13) into sterile urine, distilled water, and on blood agar slants. One set of the tubes was kept under aerobic, the other set under anaerobic, conditions at 37° C., and a blood agar slant in oxygen as well. Subcultures on blood agar plates were made March 1. The tube of blood agar kept in oxygen and the anaerobic urine tube were sterile. The others all yielded green colonies but the amount of green was distinctly less and the organisms small in the cultures from the urine. The aerobic culture in distilled water yielded 11 small hemolyzing colonies. When this strain was now grown in broth it produced a diffuse turbidity, diplococci, and short chains. It had acquired the power to ferment mannite and when injected intravenously in rabbits produced now a myositis and myocarditis in addition to arthritis and endocarditis and often focal nephritis. Its virulence was distinctly greater than before. This was manifested by the greater range of lesions produced, by its slower disappearance from the circulation, and by the fact that it

killed rabbits in smaller doses. From the pericardium of a rabbit which was injected with this strain after two animal passages, there was isolated a number of green-producing colonies. This green-producing strain was now injected intraperitoneally into guinea-pigs. Its virulence increased rapidly and after five animal passages it had acquired all the features of a moderately virulent pneumococcus. After 10 animal passages this strain had become exactly like *Str. mucosus*. The strain as isolated was also transformed into a typical pneumococcus but it required 11 animal passages. Both strains lost completely their original affinity for joints, muscles, etc., and at a certain grade of virulence produced, on intravenous injection, pulmonary hemorrhages and bronchopneumonia while still later they caused death from pneumococcemia irrespective of the place of injection.

The strain which produced green and which has been kept on blood agar now produces small dry greenish colonies. Its affinity for joints has disappeared, while its power to produce vegetative endocarditis has increased. It is quite like *Str. viridans* in every respect.

Strain 736 was isolated March 20, 1913, from the joint in a case of acute articular and muscular rheumatism. This strain resembled in every way the strain just mentioned after it had acquired slight hemolyzing powers (see Fig. 5). It produced a narrow zone of a hazy hemolysis and when injected into the ear vein of rabbits it produced myositis, myocarditis, arthritis, and endocarditis. In the joint of one rabbit in its second passage it lost the power to hemolyze and now produced green colonies instead. It did not yet ferment inulin nor produce acid in pneumonic serum. This strain had lost most of its affinity for the muscles, endocardium, joints, and kidney, and now produced acute pericarditis, acute splenitis, and a bronchopneumonia. After six passages in guinea-pigs it had all the characteristics of a pneumococcus. The hemolyzing strain during the second passage produced a moderate number of muscle lesions as well as myocarditis and endocarditis, a few in the third, but after the fourth and fifth passages the muscles, myocardium, endocardium, and kidney were no longer involved. It produced ulcer of the stomach in the second and third passages but, after subsequent passages, a suppurating arthritis instead. The strain which has been cultivated continuously on blood agar has acquired greater hemolyzing power. One month after isolation plate cultures from four of the colonies near the upper end of one of the tubes of ascites-dextrose agar, which was inoculated with joint fluid, show these organisms to be changed whereas each of the colonies in the depths yields two kinds of colonies: one similar to the colonies above and the other non-hemolyzing, more opaque, grayish colonies. Smears from these show small micrococci in clumps and an occasional short chain. This strain now resembles the third type from rheumatism. Similar results were obtained with two other strains from rheumatism.

Strain B was isolated April 18, 1912, as *Str. viridans* from the blood during life in a case of subacute infectious endocarditis. A "pure line" was obtained for further study. It produced valvular hemorrhages in five and endocarditis in two rabbits soon after isolation. After cultivation on blood agar for nearly a year it acquired the power to hemolyze blood agar, lost its affinity for the endocardium, but acquired an affinity for joints. Cultures from the joint, in one rabbit, showed both green and hemolyzing colonies. Cultures from the former were injected successively into four guinea-pigs. It now acquired a distinct capsule and the power to ferment inulin, to produce acid in pneumonic serum, and to dissolve in bile.

Strain 319 was isolated December 11, 1907, from the blood during life in a case of lobar pneumonia. It was passed through one guinea-pig at that time and the heart blood, which yielded a pure culture of a typical pneumococcus, was put away in a sealed pipette, in the dark, at room temperature for over five years. At this time a culture on blood agar yielded a pure growth of markedly hemolyzing colonies. Smears showed gram-staining diplococci and short chains. This strain as a hemolytic streptococcus had a marked affinity for joints. By growth in symbiosis with *B. subtilis* on blood agar it acquired the cultural and pathogenic features of *Str. viridans*, producing repeatedly endocarditis without arthritis. After this modified strain had been passed through 17 rabbits (intravenous injections) it resembled the green-producing strains from rheumatism and produced non-suppurative arthritis, endocarditis, and pericarditis. The eighteenth passage produced in addition a few muscle lesions, appendicitis, and a myocarditis. After 21 animal passages (the last three through guinea-pigs by intraperitoneal injection) the affinity for joints, endocardium, myocardium, and muscles was lost, and it now produced pulmonary hemorrhages when injected intravenously in rabbits, and death from pneumococcemia when injected intraperitoneally and subcutaneously. It was now a pneumococcus in every respect.

Strain R51A was isolated originally October, 1902, as a pneumococcus from the blood during life in a case of lobar pneumonia. It was passed through 51 guinea-pigs at that time and was cultivated on blood agar in sealed tubes until the fall of 1912. During this time it always produced green on blood agar. Three attempts to restore its virulence were unsuccessful, but two years ago it was found to have acquired a moderate degree of virulence for mice, rabbits, and guinea-pigs, altho an attempt to raise its virulence to a high point failed. After three generations in unsealed tubes of blood agar, the last of which had become very dry, the strain had acquired the power, for the first time in over 10 years, to hemolyze blood. A subculture from a tube which had been kept sealed still showed green non-hemolyzing colonies. Neither of these colonies fermented inulin. The hemolyzing strain was now passed through 16 rabbits (intravenous injection), and the joint culture from the sixteenth animal showed both green and hemolyzing colonies. By intraperitoneal injection of the green-producing strain successively in five guinea-pigs it acquired a capsule, the power to ferment inulin, to produce acid in pneumonic serum, and to dissolve in bile.

Strain T was isolated as *Str. mucosus* from the pus in a case of acute mastoiditis three years ago. It was passed through two guinea-pigs at that time and cultivated, with frequent transfers, for a number of months. A subculture was then made on blood agar, the tube sealed with a paraffin cork, incubated for a number of weeks and put at room temperature in the dark until two weeks ago. Subcultures now yielded an organism which resembled *Str. viridans* in every respect and on intravenous injection in rabbits it did not grow in the blood but produced endocarditis in one rabbit after three injections.

THE EFFECT OF GROWTH IN A HIGH OXYGEN PRESSURE.

The results obtained in an experiment begun on January 13, 1913, will serve to illustrate the results obtained by growing hemolytic streptococci in an atmosphere of pure oxygen. Sixteen strains of hemolytic streptococci, isolated originally from a wide range of sources, which always yielded only hemolyzing colonies,

were selected. Cultures were made on the surface of small tins, 2 cm. in diameter, containing ascites-dextrose agar. These were now placed in a sterilized five-gallon bottle. The air was displaced with oxygen by means of a tube which extended to the bottom, the bottle sealed and placed at 37° C. for 72 hours. The oxygen was washed in water and passed through a long plug of sterilized cotton in a sterile pipette. Subcultures were made in the same way on January 17 and 22. On January 25 blood agar plate cultures were made. All but seven of the strains died. Two of these yielded grayish green colonies only, none of which was adherent; three produced non-adherent gray, adherent green, as well as hemolyzing colonies, while two of the strains yielded only hemolyzing colonies. The zone of hemolysis in each, however, was no longer wide and clear but narrow and hazy. The organisms in the control cultures in tubes of ascites-dextrose agar to which no oxygen was added remained unaltered. All yielded only widely hemolyzing colonies.

Seven strains of pneumococci were treated in the same way. All lost their capsule and virulence. Three were changed so that they lost the power to produce green, and acquired hemolyzing power. All of these and two others lost the power to ferment inulin. The hemolyzing strains all produced clouding in ascites-dextrose agar. Two now produced small non-adherent colonies which did not affect the media perceptibly. Smears showed chiefly small cocci which were often in clumps and an occasional short chain. The effect of oxygenated blood and methemoglobin on pneumococci will be discussed later. In this connection the observation was made that pneumococci grow better and live longer when subject to a high oxygen pressure than hemolytic streptococci.

Three strains of *Str. viridans* were treated in the same way. One changed into the hemolyzing variety; the other two were not changed perceptibly.

THE EFFECT OF GROWTH IN SYMBIOSIS WITH OTHER BACTERIA.

Twelve of 17 strains of hemolytic streptococci were converted into *Str. viridans* by growth in symbiosis with *B. subtilis* on blood agar. Five recently isolated strains remained quite unchanged,

but after three of these strains had grown in oxygen where their hemolyzing power was reduced, on being again grown in symbiosis with *B. subtilis*, they took on cultural and other features of *Str. viridans*. Growth in broth in symbiosis with *B. subtilis* resulted positively in only one instance. Pneumococci and *Str. viridans* remain quite unchanged when grown in symbiosis with this organism. Two strains of typical pneumococci, one representing a "pure line," were changed into hemolytic streptococci by growth in symbiosis on blood agar with a hemolytic colon bacillus.

THE EFFECT OF GROWTH IN HYPOTONIC AND HYPERTONIC MEDIA.

The effect of distilled water, of ovomucoid, hypotonic and hypertonic broth, and dialyzed beef serum agar was studied on eight strains of hemolytic streptococci, five strains of pneumococci, and one strain each of streptococcus from rheumatism and *Str. viridans*.

Salt-free broth was prepared and to this varying concentrations of Ringer's solution were added. Two strains of hemolytic streptococci lost their hemolyzing power completely in hypotonic media and produced green and gray colonies. Two strains only were perceptibly modified in hypertonic media (10 and 20 times the concentration of a normal Ringer's solution). In both, the colonies on blood agar plates were extremely small but surrounded by a wide, perfectly clear zone of hemolysis. Smears of these showed small cocci and chains, similar to certain strains of streptococci isolated at times from crypts of tonsils. All the strains in distilled water and hypotonic media remained unaltered. After growth in broth made 10 times the strength of a normal Ringer's solution one strain showed a zone of hemolysis peripheral to a green colony in which corpuscles were intact, and when the concentration of salt was twice as great as this there was a clean-cut hemolysis. The strain from rheumatism was changed from a green-producer to a hemolyzer in distilled water. The *Str. viridans* remained unaltered. The effect of ovomucoid media on pneumococci is often quite striking. Typical strains are frequently so altered that instead of producing green colonies on blood agar they produce rather large gray colonies, smears showing micrococci, and only occasionally short chains which are made up of round cocci. The

TABLE 3.

FERMENTATIVE AND OTHER PROPERTIES OF VARIOUS STRAINS AS HEMOLYTIC STREPTOCOCCI AND AS *Str. viridans*.

MEDIA	STRAINS													
	L		I		713		R51A		319		683		736	
	As Hem. Str.	As Str. Vir.	As Hem. Str.	As Str. Vir.	As Hem. Str.	As Str. Vir.	As Hem. Str.	As Str. Vir.	As Hem. Str.	As Str. Vir.	As Hem. Str.	As Str. Vir.	As Hem. Str.	As Str. Vir.
Blood agar	H	G	H	G	H	G	H	G	H	G	H	G	H	G
Serum-dextrose agar. Aerobic	++	+	+	++	+	o	++	o	+	o	+	+	+	+
Serum-dextrose agar. Anaerobic	+++	++	++	+++	+++	+	+++	+	++	++	++	++	+++	++
Inulin	o	+	o	+	o	+	o	+	o	+	o	o	o	o
Pneumonic serum	o	o	−	−	o	++	o	++	o	+	−	−	o	o
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	++	+
Maltose	o	+	+	+	+	+	+	+	+	+	o	+	+	+
Saccharose	o	+	+	o	+	+	+	+	+	+	+	o	+	+
Lactose	+	+	+	o	+	o	+	+	+	+	+	+	+	+
Mannite	o	o	o	o	+	o	+	+	o	o	+	o	+	o

In Table 3 Strains L and I were originally isolated as hemolytic streptococci, Strains 713, R51A, 319, and 683 as pneumococci, and Strain 736 from a joint in rheumatism.

fermentative and other properties are also markedly changed. The morphological and other features at times resemble staphylococcus so much that controls must be made to rule out contaminations.

FERMENTATIVE POWERS OF VARIOUS STREPTOCOCCI.

Table 3 shows that when strains of hemolytic streptococci have been converted into *Str. viridans* and pneumococci into hemolytic streptococci and *Str. viridans* the modified strain in each case has acquired new fermentative powers or has lost the power to ferment one or more sugars or both. It shows further the value of blood agar media, which have been used throughout this study, as a criterion of change in the various strains. Human blood was used. Rabbit and guinea-pig blood may be used in a study of this kind, but goat and sheep blood, while they serve to differentiate typical hemolytic streptococci and pneumococci, fail to bring out characteristic differences of the intermediate strains. Dog blood is practically useless in the differentiation of these organisms because the corpuscles hemolyze readily and spontaneously.

THE EFFECT OF FILTRATES OF ASCITES BROTH CULTURES OF VARIOUS STRAINS OF STREPTOCOCCI ON THEIR GROWTH (MARMOREK'S TEST).

The organisms were grown in ascites plain broth for 48 hours, the broth filtered through Chamberland filters, cultures made to test their sterility, and the strains as indicated in Table 4 inoculated. The strain of hemolytic streptococcus (L) was isolated in pure culture from the pus in a case of long-standing empyema; the pneumococcus from the blood in lobar pneumonia; *Str. viridans* (No. 722) from the blood during life in a case of subacute infectious endocarditis, and the rheumatic strain from the joint in a case of rheumatism.

Table 4 shows that the strains of streptococci failed to grow in the streptococcus filtrates but grew in filtrates of cultures of pneumococcus, the viridans, and rheumatic streptococci. The two strains of pneumococci and the rheumatic strain failed to grow in pneumococcus filtrate but grew well in filtrates of cultures of hemolytic streptococcus, viridans, and the rheumatic streptococcus. The *Str. viridans* grew in filtrates of hemolytic streptococcus, of all but one pneumococcus, and of the rheumatic streptococcus.

The rheumatic strain (No. 736) which produced a slight hemolysis failed to grow in streptococcus filtrates while one which produced green did. This has been found to be the case with other strains.

TABLE 4.

THE EFFECT OF ASCITES BROTH CULTURE FILTRATES OF HEMOLYTIC STREPTOCOCCI, PNEUMOCOCCI, "STR. RHEUMATICUS," AND *Str. viridans* ON THE GROWTH OF THESE ORGANISMS.

FILTRATES	STRAIN INOCULATED										
	Streptococcus L	Pneumococcus S	Pneumococcus 678[19]	XII[2] As a Streptococcus	XII[2] As a Pneumococcus	595 As a Streptococcus	595 As a Pneumococcus	"Str. rheumaticus" 736	734 As a Streptococcus	734 As a Pneumococcus	722
Hemolytic streptococcus (L)	o	+	+	o	+	o	+	o	+	+	+
Pneumococcus	+	o	o	+	o	+	o	+	o	+	+
Strain XII[2] as a pneumococcus	+	o	o	+	o	+	o	+	+	o	+
Strain 595 as a pneumococcus	+	o	o	+	o	+	o	+	+	o	o
Str. viridans (722)	+	+	+	+	+	+	+	+	+	+	o
"Str. rheumaticus" (734)	+	+	+	+	+	+	o	+	+	+	+
Ascites-plain broth (control)	+	o	+	+	+	+	o	+	+	+	+

It is thus seen that the strains made over into pneumococci behave as do other strains of pneumococci toward filtrates of hemolytic streptococci and of pneumococci.

SUMMARY OF CHARACTERISTICS OF VARIOUS STRAINS OF STREPTOCOCCI AND PNEUMOCOCCI.

In Table 5 is given the morphology and the behavior of various strains which were originally either hemolytic streptococci, "*Str. rheumaticus*" or pneumococci, toward the various tests used in differentiating these organisms. These tests were made after the transformation of one into the others was seemingly complete. During the transition stages the cultural tests fail to give characteristic reactions and it is often difficult to know where an organism belongs, but after the organisms, which have the morphology, cultural, and pathogenic properties of hemolytic streptococci, have been converted into *Str. viridans* and then have acquired a capsule and high virulence from animal passage, they react like pneumococci in every respect. In order to satisfy myself further that the streptococci which I had converted into pneumococci were really streptococci and those transformed really pneumococci,

TABLE 5.

SUMMARY OF THE MORPHOLOGY, CULTURAL AND OTHER PROPERTIES OF VARIOUS STRAINS AS STREPTOCOCCI AND AS PNEUMOCOCCI.

STRAIN	MORPHOLOGY, CULTURAL AND OTHER PROPERTIES													
	As a Streptococcus							As a Pneumococcus						
	Morphology	Blood Agar Plates	Clouding of Serum Dextrose Agar	Fermentation of Inulin Broth	Acid in Pneumonic Serum	Solubility in Bile	Autolysis in NaCl Solution	Morphology	Blood Agar Plates	Clouding of Serum Dextrose Agar	Fermentation of Inulin Broth	Acid in Pneumonic Serum	Solubility in Bile	Autolysis in NaCl Solution
595	Diplococci and short chains....	H	++	o	o	o	o	Encapsulated, often lance-shaped diplococci.....	G	o	++	++	+	+
XII[2]	Diplococci and rather long chains	H	+	o	o	o	o	Encapsulated diplococci and short chains.....	G	o	+	++	++	+
735	Diplococci and short chains....	H	+++	o	o	o	o	Widely encapsulated diplococci..	G	o	+	+	++	+
736	Diplococci and short chains....	H	+++	o	o	o	o	Encapsulated, often lanceolate diplococci.....	G	o	++	++	+	+
319	Diplococci, short chains, and small clumps.....	H	+	o	o	o	o	Encapsulated, often lanceolate diplococci.....	G	o	+	++	+	+
R51A	Diplococci and chains.....	H	++	o	o	o	o	Encapsulated, often lanceolate diplococci.....	G	o	++	+	++	+
734	Diplococci and short chains.. .	H	+++	o	o	o	o	Encapsulated diplococci	G	o	++	++	+	+
678	Diplococci and short chains*...	H	++	o	o	o	o	Encapsulated, often lance-shaped diplococci.....	G	o	++	+	+	+
2.18[17]	Diplococci and chains.....	H	++	o	o	o	o	Encapsulated, lanceolate diplococci.....	G	o	+	++	++	+

*With the exception of Strain 678, there were no capsules

I felt that their identification by an independent observer was highly desirable. Through the kindness of Dr. Libman I was able to do this. A series of 10 cultures containing known hemolytic streptococci, *Str. viridans*, and pneumococci, together with strains as streptococci and as pneumococci, was sent to him as unidentified cultures. They were studied in the laboratories of Mt. Sinai Hospital, New York, and Dr. Libman's identification agreed with mine in every respect.

EXPERIMENTS ON AGGLUTINATION.

Cole and his co-workers would divide pneumococci into four groups according to the reactions of agglutination and the specific protection of the immune serum.[1] As this division appears to hold good for pneumococci as they occur in pneumonia it no doubt marks an advance in the efforts to secure effective antipneumococcal serum. Specific sera have been secured by Cole and his associates for Groups I and II which contain the types of pneumococci most frequently encountered in pneumonia, the cases caused by Group II being especially severe and associated with a moist pneumonic exudate.

In view of these considerations the results of observations on a pneumococcus culture, seemingly of Group I, are of interest.

Pneumococcus 678 was isolated from the blood of a pneumonia patient October 25, 1912, and after two transplantations on blood agar a culture was secured from a single coccus by Dr. V. H. Moon. The colonies of this pure line were typically green; the organism fermented inulin, produced acid in pneumonic serum, dissolved readily in salt solution and in bile and sodium oleate. Growth continued typical of pneumococcus on blood agar in closed tubes, but the capsule was lost. After passage through 22 guinea-pigs, the virulence, the capsule, and the growth increased. Spreading colonies developed on blood agar plates similar in every way to the colonies from pneumonic lungs with a slimy exudate.

Inoculations (before the guinea-pig passage) on ascites-dextrose agar in pure oxygen resulted after three transplantations in loss of virulence and at the same time in a gain of hemolytic power. After passing through 15 rabbits, the injections being intravenous, the organisms of this strain continued hemolytic, were not affected by salt solution or bile, and did not ferment inulin nor produce acid in pneumonic serum.

On June 24, 1913, the original strain that remained as a pneumococcus, and the strain after animal passage, produced green colonies only on blood agar; and the hemolyzing strains produced only colonies with typical zones of hemolysis. Agglutination tests of these various strains were now made with Dr. Cole's Sera I and II (Table 6).

[1] *Jour. Am. Med. Assn.*, 1913, 51, p. 663; also Dochez and Gillespie, *ibid*, p. 727.

TABLE 6.

VARIATIONS IN AGGLUTINATION OF STRAIN 678 AS PNEUMOCOCCUS AND AS STREPTOCOCCUS.

SERUM	AS PNEUMOCOCCUS		AS STREPTOCOCCUS	
	Before Passage	After Passage	Before Passage	After Passage
I	+	o	o	o
II	o	++	o	o

For Antipneumococcus Sera I and II, I am indebted to Dr. R. I. Cole, Director of the Hospital of the Rockefeller Institute for Medical Research, New York. These sera have strong agglutinating powers.

The mixtures were made by adding 0.5 c.c. of serum to 0.2 c.c. of the washed suspension of cocci in salt solution (in each case approximately one-sixth of a blood agar slant). The tubes were incubated at 37° C. for two hours and then placed at room temperature.

We have here a pneumococcus which would be classed in Group I when isolated from the blood of the pneumonia patient, but after passing through guinea-pigs, the agglutination places in Group II, while the streptococcus mutations practically are not agglutinated at all.

A similar experiment made on a large scale by using six different cultures gave the results recorded in Table 7. Two of the cultures, Strains 595 and XII[2], were originally hemolyzing streptococci; two, Strains 734H and 736, were isolated from the joints in rheumatism; and two, Strains 319 and 678, were originally pneumococci.

TABLE 7.

AGGLUTINATION TESTS OF VARIOUS STRAINS AS HEMOLYTIC STREPTOCOCCI AND AS PNEUMOCOCCI.

SERUM	595		XII[2]		734H		319		736		678	
	As Streptococcus	As Pneumococcus	As Streptococcus	As Pneumococcus	As Streptococcus	As Pneumococcus	As Streptococcus	As Pneumococcus	As Streptococcus	As Pneumococcus	As Streptococcus	As Pneumococcus
Antipneumococcus I	o	o	o	o	o	o	o	++	o	o	o	+
Antipneumococcus II	o	++	o	++	o	++	o	++	o	++	o	o
Antipneumococcus M	o	+	o	+	o	+	o	+	o	+	o	o
Antistreptococcus M	+	o	+	o	o	o	+	o	+	o	+	o

Antipneumococcus Serum M and Antistreptococcus Serum M were obtained from Mulford and Co. through the courtesy of Dr. Hitchens.

We see that as streptococci they are with one exception agglutinated by antistreptococcus serum but not by antipneumococcus serum, while as pneumococci they are agglutinated by antipneumococcus serum only and with one exception most strongly by Antipneumococcus Serum II. The strains which have all the other

features of streptococci behave as streptococcci and the strains which are like pneumococci in other respects behave like pneumococci with respect to agglutinating serum.

That pneumococci easily are made to change their agglutinative properties is shown also by some experiments I made with cultures of Pneumococcus, Groups I and II, which Dr. Cole kindly sent me. After growing the organisms on dextrose blood agar, ascites-dextrose agar and broth, and in ascites broth and washing them in NaCl solution I found that only a degree of specific agglutination occurred. Pneumococcus I was agglutinated by Serum I when grown in all media except ascites-dextrose agar; the organisms from this medium were agglutinated by Serum II. Pneumococcus II was agglutinated specifically when grown in ascites-dextrose broth; it was agglutinated by Sera I and II when grown on the other media.

These results are in accord with those of Bordet and Sleeswijk[1] and Gay and Claypole,[2] the former demonstrating variations in the agglutinability of the bacillus of whooping cough, the latter inducing variations in the agglutinability of the bacillus of typhoid fever.

TRANSFORMATION OF PNEUMOCOCCUS GROUPS I AND II INTO STREPTOCOCCI.

Through the kindness of Dr. Cole I have been enabled to experiment with his Pneumococci I and II, the cultures used by Dr. Cole and his associates to develop Antipneumococcus Sera I and II. Pneumococcus I was isolated originally by Professor Dr. Neufeld.[3]

In order to subject the possibility of transformation of pneumococci into streptococci to a supreme test I therefore subjected cultures of Pneumococci I and II to a wide range of conditions. Altogether Pneumococcus I has been passed through 70 animals, Pneumococcus II through 16. Both were highly virulent and resistant to phagocytosis, and corresponded to typical pneumococci in every respect. Pneumococcus II, however, produced the

[1] *Ann. de l'Inst. Pasteur*, 1910, 24, p. 476.

[2] *Jour Am. Med. Assn.*, 1913, 60, p. 1141.

[3] Neufeld and Haendel, *Arb. a. d. k. Gsndhtsamte*, 1910, 24, p. 293.

moister growth and its capsule was distinctly wider and more easily stained.

As pointed out it is difficult to produce mutations of highly virulent pneumococci because they usually die before important changes take place. After suspending growths of Pneumococci I and II in hypertonic Ringer's solution, in distilled water, and after cultivation in ovomucoid media, in sterilized urine, in plain broth, in broth to which sodium iodbenzoate and sodium iodoxybenzoate had been added, clean-cut hemolyzing colonies were not obtained on blood agar altho in some colonies hemolysis was separated from the colony itself by a zone in which the corpuscles were intact. Blood agar plates were inoculated every day at first and then every other day until the tubes became sterile.

A medium in which virulent pneumococci live longer when subjected to changes in oxygen pressure was found in sterile defibrinated blood. The oxygen tension could be made high or low as desired by shaking with oxygen or carbon monoxid gas.

On July 11, Pneumococci I and II and two other virulent pneumococci (Nos. 678 and 734) were inoculated each into two eight-ounce bottles containing 2 c.c. of sterile human blood. In each case the air in one bottle was displaced by oxygen, in the other by illuminating gas (carbonic oxid). The gases were washed and passed into the bottles through long well-fitting cotton plugs in sterile pipettes, the bottles tightly closed with rubber stoppers and sealed with paraffin. They were now placed in a shaker at 37° C. for 24 hours. Inoculations were now made from the bottles which were put in diffuse sunlight at room temperature until July 20, inoculations being made on blood agar plates in 3, 5, 8, and 14 days. Pure cultures of green colonies in diminishing numbers were obtained in each instance until the fourteenth day, the number of visible organisms per loop now having dropped to under 100 in each of the bottles containing oxygen whereas in those containing the gas the number was higher. Pneumococcus II and Pneumococcus 678 in the bottles containing oxygen gave a number of fine colonies surrounded by hemolysis, but the hemolysis was outside of a zone in which the corpuscles were intact. On July 30 one of these bottles gave 11, and the other, five colonies which produced a clear zone of hemolysis that began immediately outside the colony itself. At the same time the oxygen bottle containing Pneumococcus II gave nine green colonies, and the oxygen bottle with Pneumococcus 678 gave 53.

Pneumococcus I was lost in the oxygen treated blood, while from the blood saturated with carbonic oxid was obtained a number of colonies, none showing any noteworthy change.

Inoculations on blood agar plates from four of the hemolyzing colonies of Pneumococcus II and from two of Strain 678 yielded only hemolyzing colonies.

Two similar experiments, in which different samples of blood were used, were made on August 6 and 15. Strains 734H, 736, and XII, which had been made into

pneumococci, were included. In only two instances were hemolyzing colonies obtained from the blood saturated with carbonic oxid. Two of the mutant strains, Nos. 736 and XII, yielded hemolyzing colonies from the oxygen treated blood in both experiments; the other, Strain 734H, in only one. Pneumococcus II yielded hemolyzing colonies again in the experiment begun August 15 but not in the one begun August 6. Pneumococcus I proved most refractory, but yielded seven hemolyzing colonies on the thirteenth day in the experiment begun August 15 together with 23 green colonies. Hemolyzing colonies were obtained also on the following day. Plate inoculations from four of these colonies yielded hemolyzing colonies only.

The results of further tests with the original and modified organisms are given in Table 8.

TABLE 8.

CHARACTERISTICS OF PNEUMOCOCCI I AND II AS PNEUMOCOCCI AND AS STREPTOCOCCI.

STRAIN	CAPSULE	AGGLUTINATION BY ANTIPNEUMOCOCCUS SERUM	SUSCEPTIBILITY TO PHAGOCYTOSIS	ACID IN PNEUMONIC SERUM	CLOUDING OF ASCITES-DEXTROSE AGAR	FERMENTATION OF CARBOHYDRATES				AUTOLYSIS IN NACL SOLUTION	SOLUBLE IN BILE
						Inulin	Mannite	Raffinose	Saccharose		
I as Pneumococcus...	+	+	o	+	o	+	o	++	+	+	++
I as Streptococcus ...	o	o	+	o	++	o	++	o	+	o	o
II as Pneumococcus..	+	+	o	+	o	+	o	+	+	+	++
II as Streptococcus ..	o	o	+	o	+	o	+	o	+	o	o

The difference in the behavior of the organisms when treated with bile was striking. The growth from blood agar was washed once in NaCl solution and bile then added. The pneumococcal form of the organisms dissolved in 15 minutes whereas the streptococcal forms remained gram-positive for 48 hours.

The agglutination reactions of various cultures of Pneumococci I and II were now determined. The results are given in Table 9.

A slight clumping of the streptococcal forms was observed at the end of 24 hours in Antipneumococcus Sera I and II. A diffuse turbidity, however, was still present and the sediment easily broken up by shaking. Similar sediments were obtained in the case of organisms grown continuously on blood agar when Pneumococcus I was acted on by Serum II and Pneumococcus II by Serum I. This was not considered a true agglutination, but to satisfy myself further on this point the effect of Sera I and II was tested on five typical strains of hemolytic streptococci. The clumping of the streptococci at the end of 24 hours was comparable

to that of I and II as streptococci. In Table 7, Strains 595, XII, 734H, and 736 as pneumococci were agglutinated most strongly by Serum II; after treatment in highly oxygenated blood in which four of the strains yielded hemolyzing colonies, which were no longer

TABLE 9.

AGGLUTINATION OF PNEUMOCOCCI I AND II AS PNEUMOCOCCI AND AS STREPTOCOCCI.

STRAIN	SERUM							
	Antipneumococcus Serum I		Antipneumococcus Serum II		Antipneumococcus Serum M		Antistreptococcus Serum M	
	5 hr.	24 hr.	5 hr.	24 hr.	5 hr.	24 hr.	5 hr.	24 hr.
I Cultivated continuously on blood agar..	Complete	Solid coagulum	o	o	o	Loose coagulum	o	o
II Cultivated continuously on blood agar..	o	o	Cómplete	Solid coagulum	o	Loose coagulum	o	o
I While in oxygen but still a pneumococcus.	Complete	Coagulum less firm	Beginning	Complete	o	+	o	o
II As a streptococcus..	o	o*	o	o*	o	o	Beginning	Complete
II While in oxygen but still a pneumococcus.	Beginning	Complete	Marked	Solid coagulum	o	+	o	o
II As a streptococcus...	o	o*	o	o*	o	o	o	Complete

* See statement in text, p. 25.

Same technic as in experiment recorded in Table 6.

agglutinated by antipneumococcus serum, two of the strains (Nos. 595 and 736), still growing as pneumococci, were agglutinated by Serum I instead of Serum II while the other two were agglutinated equally well by both.

In Table 10 are given the results of the agglutination by various dilutions of Serum I of Pneumococci I and II as pneumococci and as streptococci. The result is clear and needs no discussion. The mixtures were kept at 37° C. for five hours, then at room temperature.

The effect of the organisms on rabbits and mice was next studied. The original cultures killed mice after intraperitoneal injection, and rabbits after intravenous injections, with a rapid pneumococcemia. On the other hand the mice injected with comparable doses of the streptococcal modification recovered, and peritoneal smears 5 and 15 hours after injection showed marked

phagocytosis. Rabbits were injected with each of the streptococcal forms and also with comparable doses (one-fourth blood agar slant) of the original pneumococcus cultures. The two injected with the pneumococcus culture died in 24 hours with pneumococcemia; both showed marked pulmonary hemorrhages, hemorrhagic enteritis and acute splenitis, the joint fluid remaining clear. The rabbits injected with the streptococcal forms remained

TABLE 10.

THE EFFECT OF DILUTING SERUM I ON ITS AGGLUTINATING POWER OVER PNEUMOCOCCI I AND II AS PNEUMOCOCCI AND AS STREPTOCOCCI.

STRAIN	AGGLUTINATING POWER OF VARIOUS DILUTIONS OF SERUM I											
	1–10			1–30			1–90			1–270		
	1 hr.	5 hr.	48 hr.	1 hr.	5 hr.	48 hr.	1 hr.	5 hr.	48 hr.	1 hr.	5 hr.	48 hr.
I As a pneumococcus....	+	++	++	+	+	++	o	Beginning	++	o	o	o
I As a streptococcus	o	o	Slight	o	o	Slight	o	o	o	o	o	o
II As a pneumococcus...	o	Slight	Solid coagulum	o	o	+	o	o	o	o	o	o
II As a streptococcus....	o	o	Slight	o	o	o	o	o	o	o	o	o

apparently well after the first injection, but succumbed in from two to five days after an additional and larger injection. In both, the joint fluid was markedly turbid and contained hemolyzing streptococci. One rabbit had an "ascending" nephritis, and one a few muscle lesions; neither had endocarditis nor pericarditis. To study further the changed pathogenic power, on August 15 two rabbits were injected with larger doses of the streptococcal growths. The record of one of these rabbits is given.

August 16. Rabbit seemed quite well; knee joints punctured. Turbid fluid with leukocytes and diplococci. Cultures of joint fluids and blood on blood agar plates.

August 17. Blood sterile; joint fluids showed large number of hemolyzing colonies in pure culture.

August 19. Lame in right hind leg. Knee joint swollen and tender.

August 21. Found dead. Cloudy swelling of heart and kidneys, no distinct focus of infection in the kidney; acute cholocystitis; gall bladder distended with a bile-stained mucus in which were flakes of leukocytes and many diplococci and streptococci; in the wall of the gall bladder were a number of small, grayish nodules which

projected on the inner surface and involved chiefly the mucous membrane. On transmitted light it was found that these nodules occurred in the parts farthest removed from the blood vessels. Smears from these areas, the tissue of which was washed and then crushed, showed leukocytes and gram-staining diplococci in short chains. Paraffin sections of the gall bladder showed streptococci in these areas. Right knee joint distended with turbid, thick fluid, with many leukocytes, endothelial cells, and a few diplococci. One small streak in the subscapular muscle. Stomach, appendix, liver, and spleen normal. Cultures on blood agar plates.

August 23. Cultures; blood sterile; crushed tissue from gall bladder and bile gave large number of hemolyzing colonies only; joint fluids yielded three green and no hemolyzing colonies; pelvis of kidney, a few hemolyzing colonies.

It is thus shown that as the morphology, cultural, and agglutinating properties of the two strains change, the pathogenic properties change likewise. Just as in the strains isolated by me they act in every way like certain strains of hemolytic streptococci. From these results it appears that while pneumococci under certain conditions may be divided into distinct groups by means of agglutination and other reactions, a change of environment may produce radical changes in agglutinative as well as other properties. The fact that they may even be converted into streptococci is, nevertheless, no reason why varieties of pneumococci may not be found in pneumonia, for example, as worked out by Cole and his associates, but to consider the varieties as "fixed" races hardly seems warranted.

SUMMARY.

Altogether 21 strains isolated originally as hemolytic streptococci from a wide range of sources, including erysipelas, scarlet fever, puerperal sepsis, arthritis, tonsillitis, cows' milk, etc., have in one way or another been converted into *Str. viridans;* 3 into *Str. viridans* and into typical pneumococci, and 1 into *Str. mucosus* as well; one of these corresponded at one time to the streptococci from rheumatism.

Seventeen strains which were isolated as *Str. viridans* chiefly from the blood and tonsils in cases of chronic infectious endocarditis and two strains from cows' milk have been converted into pneumococci and 2 of these into *Str. mucosus* also; 10 have been made to take on cultural and morphologic characteristics of hemolytic streptococci, in 2 of which the pathogenic powers were

shown to be those of hemolytic streptococci; 1 strain was converted into hemolytic streptococcus, into *Str. viridans*, and into a pneumococcus.

Eleven strains isolated as pneumococci from the sputum, blood, and lung in pneumonia, and from empyema and Cole's Strains I and II have been made to correspond to hemolytic streptococci; 7 took on the features of *Str. viridans;* the streptococci from 3 of these strains by animal passage acquired all the essential features of the streptococci of rheumatism; 2 have been converted into hemolytic streptococcus, the streptococci of rheumatism, *Str. viridans*, and back again into pneumococcus.

Five strains of *Str. mucosus* have taken on the cultural features of hemolytic streptococci. Two of these were converted into *Str. viridans*.

Five strains of the streptococcus of rheumatism have taken on the features of hemolytic streptococci, 2 of *Str. viridans*, and 4 have been converted into pneumococci.

In order to meet the objection that even tho every ordinary precaution was taken to obtain pure cultures, I was working with mixtures whenever mutation was observed, cultures of each main variety were obtained from single organisms by the Barber method. The same results were obtained with three of these "pure line" cultures of hemolytic streptococci, 6 of *Str. viridans*, and 2 each of *Str. mucosus* and pneumococcus. Hence the changes observed are not due to mixtures nor to so-called "mass selection" but to actual changes wrought under the influence of changed environment.

The transformation of some of the strains has been found to be complete by every test known. Thus the morphology, the presence of capsule, the fermentative powers, the solubility or insolubility in bile and in NaCl solution, the behavior toward the respective broth culture filtrates (Marmorek's test), the specific immunity response, as manifest by the production of opsonin and agglutination by antipneumococcus and antistreptococcus serum, and the more or less specific pathogenic powers have been studied. Strains that corresponded to hemolytic streptococci have been converted into typical pneumococci as determined by all the above tests and vice versa.

Now that the various strains of the streptococcus group may be converted each one into the others the question of nomenclature comes up. For the present the names which have been used in the past must be continued because convenient and because it is at these points that they show certain distinctive morphological, cultural, and pathogenic properties.

The results obtained show clearly why the classification of streptococci based on fermentative powers alone has proven to be unsatisfactory.

The changes observed have frequently the characteristics of true mutations because they appear suddenly, under conditions more or less obscure and because the newly acquired properties persist unless the organisms are again placed under special conditions. A pre-mutational stage seems to be necessary because the same strain will not yield mutants when placed under what seem to be identical conditions at different times. The underlying conditions which tend most to call forth changes are, first, favorable conditions for luxuriant growth and then unfavorable conditions—under stress or strain. This seems to call forth new or latent energies which were previously not manifest and which now have gained the ascendency and tend to persist. This may hold true *in vivo* also. This fact makes it difficult to obtain mutations outside of the body with highly virulent strains, because they die before there is opportunity for the organisms to adjust themselves to the new conditions. It explains also why injection into cavities makes for greater changes than intravenous injections of moderately virulent organisms. Apparent mutations in animals have been observed almost exclusively in closed cavities, such as joints and pericardium, and here mostly when the tissues of the host were gradually getting the upper hand and the organisms were being destroyed. The mutations *in vitro* may be spoken of as "retrogressive" and those observed in animals as "progressive" because in the former virulence, fermentative powers, and other evidences of a vigorous vegetative life are diminished whereas in the latter they are usually increased.

The bearing these results have on bacteriology, epidemiology, and medicine might be discussed at length; only the following

point will be mentioned: the fact that variations in oxygen tensions, and salt concentration, that growth in symbiosis with other bacteria and that injections into cavities in animals commonly call forth mutational forms in streptococci suggests strongly that similar changes might occur in various foci of infection where such conditions may prevail. It would seem, therefore, that focal infections are no longer to be looked upon merely as a place of entrance of bacteria but as a place where conditions are favorable for them to acquire the properties which give them a wide range of affinities for various structures.

From this study the apparent position of the various members of the streptococcus group may be illustrated by the position of the fingers in a partially flexed hand, in which hemolytic streptococcus ccupies the position of the little finger, the pneumococcus the place of the index finger (the opposite extreme), *Str. viridans* (representing the group of more or less saprophytic, non-hemolyzing streptococci) the middle finger, the streptococci from rheumatism the fourth finger, and *Str. mucosus*, having some of the properties of both pneumococci and streptococci, the position of the thumb. In this grouping there is in general an increase in parasitism and virulence as we approach the thumb (*Str. mucosus*). Being members of the same family, the sign of reversible chemical reaction ($\rightleftarrows$) between each might be used to indicate their transmutability.

EXPLANATION OF PLATE 1.

The magnification of the microphotographs in each figure is 1,200 diameters. Rosenow's capsule stain was used. The apparently large size of the strains as pneumococci in the figures is due in a large measure to fixation and the amount of decolorization. When organisms are free from capsules as well as in those portions of the smear where the capsule is not stained, in strains which have capsules, they shrink during fixation with tannic acid. The larger the capsule the larger the organisms appear to be. This fact explains the differences and the apparently larger size of the pneumococci in Figs. 3, 6, and 7.

FIG. 1.—Strain 595 as a hemolytic streptococcus, isolated from a case of scarlet fever. Smear from 24-hour culture in ascites-dextrose broth. Gram stain.

FIG. 2.—Strain 595 as *Str. viridans*. Smear from 24-hour culture in ascites-dextrose broth. Gram stain.

FIG. 3.—Strain 595 as a pneumococcus. Smear from 24-hour culture in ascites-dextrose broth. Capsule stain.

FIG. 4.—Strain of streptococcus from rheumatism which produced slight hemolysis on blood agar and myositis in animals. Smear from blood agar slant. Capsule stain.

FIG. 5.—The same strain as Fig. 4 after it is transformed into a pneumococcus. Smear from blood agar slant. Capsule stain.

FIG. 6.—Highly virulent pneumococcus (Group I) isolated originally by Neufeld and sent to me by Dr. Cole. Smear from surface and water of condensation of blood agar slant. Capsule stain.

FIG. 7.—The same strain as in Fig. 6 after being transformed into a hemolytic streptococcus. Smears from the surface and water of condensation of blood agar slant. Capsule stain.

PLATE 1.

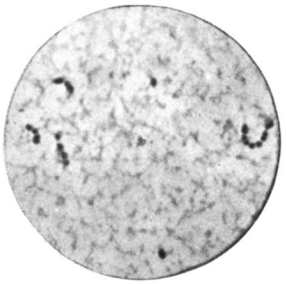

FIG. 1.

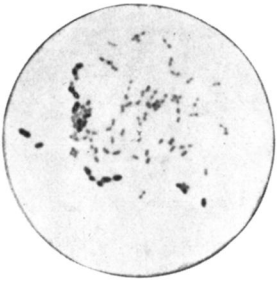

FIG. 2.

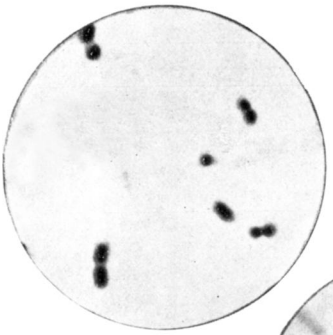

FIG. 3.

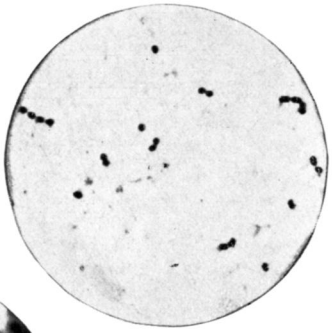

FIG. 4.

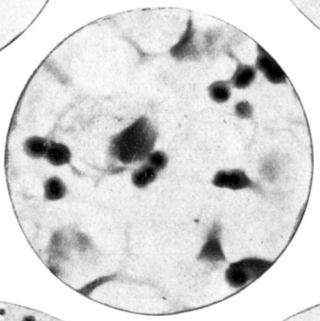

FIG. 5.

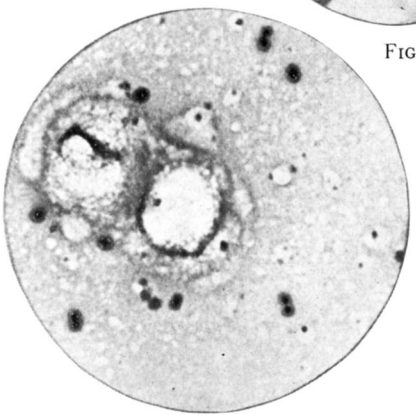

FIG. 6.

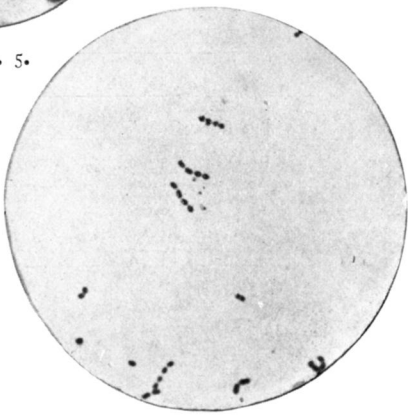

FIG. 7.